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Statolith Length and Increment Number for Age Determination of *Illex illecebrosus* (Lesueur, 1821) (Cephalopoda, Ommastrephidae)

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Abstract

A new method of counting growth increments in the statoliths of *Illex illecebrosus* is described. The increments were found to increase in number with length of statolith and dorsal mantle length of the squid. However, statolith length was found to vary less than number of increments in individual samples and may prove to be a better indicator of age. The increment-bearing portion of the statolith can be divided into three regions on the basis of increment widths and their variation. These regions reflect different stages of development of the animal. Larval retention of gross morphological characteristics, and changes in growth pattern and configuration of the statolith indicate that the larval condition extends beyond the rhynchoteuthion stage (about 8 mm dorsal mantle length) and may include squid up to 50 mm.

Introduction

Recent interest in resource management of short-finned squid (*I. illecebrosus*) has stimulated efforts toward age determination. Previous attempts to age this squid have been made by using length distributions (Squires, 1967; Mesnil, 1977) and statoliths (Hurley *et al.*, MS 1979; Hurley and Beck, MS 1980; Lipinski, MS 1980) with varying degrees of success. Those studies were limited chiefly by the scarcity or absence of very young specimens. In the present study, the size of statoliths and the number of growth increments in them were investigated in an effort to determine the age, and, by extrapolation, the date of hatching of the squid.

Microscopic patterns of lamellae in the statoliths are variously referred to as "growth rings", "growth lines" or "growth increments". The definition in this paper parallels those of Pannella (1980), who defined a "growth ring" as a single dark lamella and a "growth increment" as a light lamella plus the immediately following dark lamella. For counting purposes, "ring" and "increment" are essentially synonymous, but they must be considered as different when referring to the physiology of their formation.

Materials and Methods

The squid were collected during 1981 by midwater trawl at four offshore sites south of Newfoundland and by Japanese jigging machine at an inshore site (Come by Chance) in Placentia Bay (Table 1) and were frozen in plastic bags. In the laboratory, the samples were thawed with the bags submerged in lukewarm water.

TABLE 1. Capture information pertinent to the samples of short-finned squid used in this study of statoliths.

Date (1981)	General location	Position	Depth (m)	Gear[a]	No. of Squid	Size range (mm ML)
27 Feb	Gulf Stream	38°25'N 56°00'W	100	MWT	10	13-29
04 Mar	Gulf Stream	41°15'N 53°00'W	100	MWT	3	21-30
25 May	Carson Canyon	45°00'N 45°30'W	500	MWT	5	109-118
20 Jun	S. Grand Bank	43°36'N 51°55'W	124	MWT	52	130-191
13 Oct	Placentia Bay Newfoundland	47°45'N 51°01'W	10	JJM	42	225-266

[a] MWT = Engel midwater trawl; JJM = Japanese jigging machine.

The squid were measured as dorsal mantle length (ML) to the nearest millimeter.

Upon dissection of cartilage in the cephalic region, statocysts were cut open and the statoliths were removed with fine-pointed forceps. Adhering tissue and other debris were removed by dipping the statoliths in 5% sodium hypochlorite. The statoliths were then washed twice in distilled water and stored in 100% glycerine. At the time of examination, the statoliths were mounted in glycerine on glass microscope slides, and measurements were made with a micrometer in the eyepiece of a Nikon S-Ke II microscope. The nomenclature of Clarke (1978) was used for the identifiable parts of a statolith (Fig. 1).

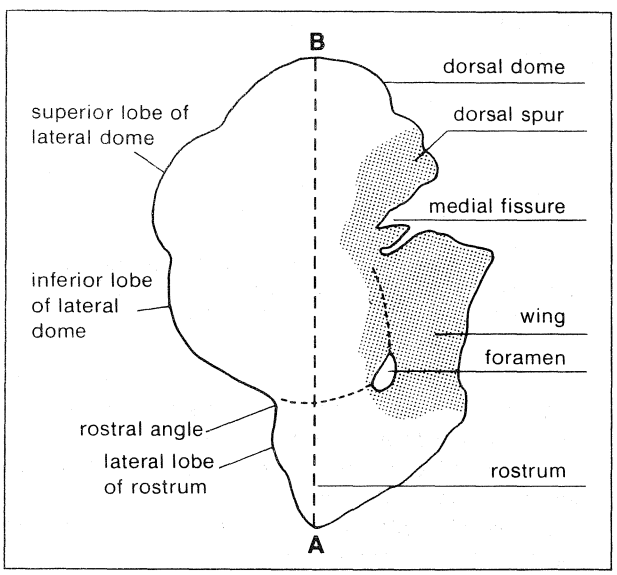

Fig. 1. Diagram of an *I. illecebrosus* statolith, showing the various aspects of its structure and the axis of measurement. (Dotted line indicates position of posterior dome indentation, and strippled area indicates region of irregularly-arranged crystals.)

After the statoliths were washed in 95% ethanol to remove the glycerine, they were rinsed twice in distilled water, air-dried, and mounted individually with convex side down in single drops of EPON 812 resin (Fisher Scientific Company) on glass slides. The slides were then placed in a polymerizing oven for 8–12 hr at 50° C. The success of observing increments over the counting areas is greatly improved if one ensures that orientation of the statoliths has not changed during processing and that the dorsal dome of each projects slightly downward.

To facilitate observation of increments, the mounted statoliths were ground against a glass plate in a mixture of 1200 grit carborundum powder and glycerine and were stained with ninhydrin-schiff according to the method of Humason (1976). With the use of a Zeiss microprojector, an image of each statolith was projected onto an aluminum surface-coated mirror and reflected on the whitest paper available. Increments were traced on the paper, the width of each being represented by the width of a pencilled line and the darkness by the length of the line (Fig. 2). Three tracings of each statolith were made before the increments were counted. The accepted count was taken as the average of the two most similar counts which differed by no more than 5%. If these conditions were not met, a fourth tracing was made.

The first trials of grinding revealed an area of irregularly-arranged crystals near the nucleus of the

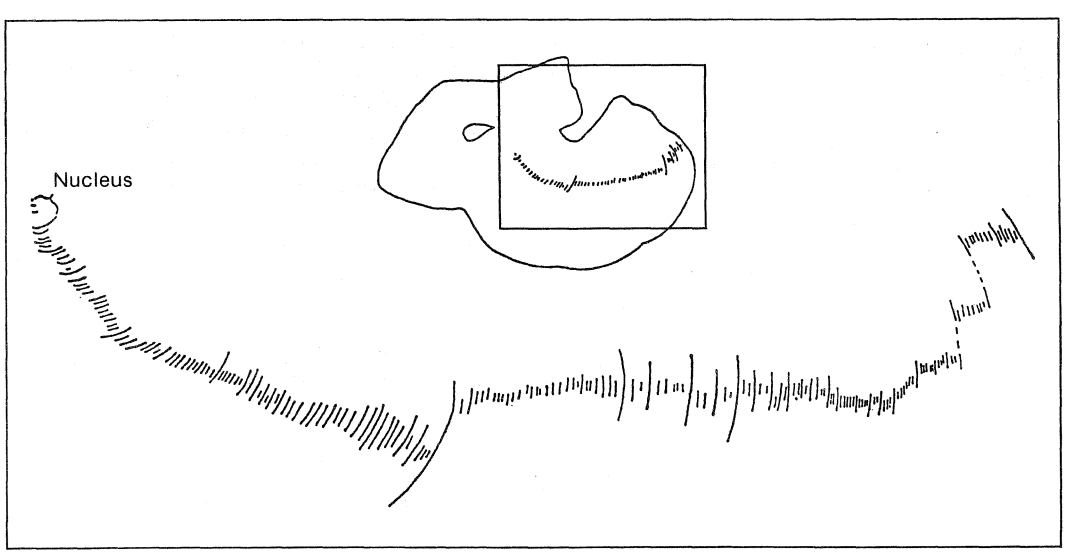

Fig. 2. Tracing of rings observed in a ground and stained statolith from a female *I. illecebrosus*. (Length of line represents relative darkness, and thickness of line indicates width or ring.)

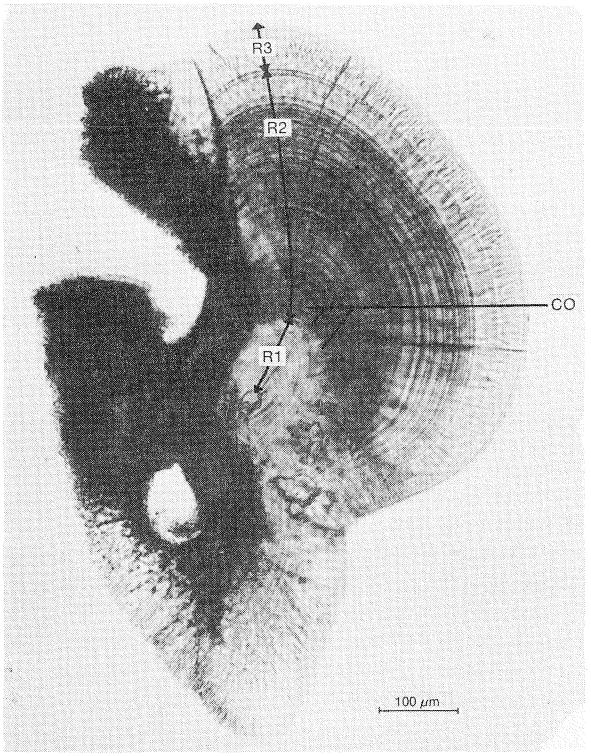

Fig. 3. Ground surface of a statolith from a male *I. illecebrosus* (168 mm ML), showing the three regions (R1, R2 and R3) of increment formation and position of the occulting crystals (CO).

statolith (see Fig. 3). These crystals occluded the underlying increments. Many of the crystals, but not all, could be removed during grinding if the dorsal dome is properly positioned. This is best done midway through the polymerizing process, when the resin is still pliable but sufficiently hard to anchor the statolith.

Statoliths from male *I. illecebrosus* were more difficult to prepare for analysis than those from females, as has been pointed out by Lipinski (MS 1980). The data in this paper are for female squid only, because statoliths from the male specimens were damaged or lost during the course of developing and perfecting the methodology that was used.

Results and Discussion

Analysis of data on increment size and number indicated that the statolith of *I. illecebrosus* can be divided into a nucleus and three regions (Fig. 3, Table 2). The nucleus consists of an inner translucent area (kernel) and the initially-formed ring. The first region (R1) outside the nucleus contains relatively narrow and uniform increments. The second region (R2) comprises the major increment-bearing portion of the statolith and is characterized by increments that are more variable in width and darkness but generally wider than those in R1. At the inner margin of R2, the main axis of statolith growth shifts about 35 degrees toward the dorsal midline of the animal (Fig. 3). The third region (R3), containing narrow regularly-spaced increments, is the last region to be formed and was observed only in female squid which, upon examination, were found to have enlarged ovaries, indicating the onset of sexual maturity.

From the curvilinear nature of the points in Fig. 4, it seemed appropriate to fit the von Bertalanffy growth relationship

$$L_t = L_\infty[1 - e^{-K(t-t_o)}]$$

where L_t is the number of increments (or statolith length) at age t (months), L_∞ is the asymptote of the growth curve, K is the growth coefficient, and t_o is the arbitrary origin of the growth curve at $L_t = 0$. Calculation of the instantaneous rate of increment formation in late February (Fig. 4A), by the equation SLOPE = $K(L_\infty - L_t)$, yields an approximate rate of 27.6 increments per 30-day month (0.93 increments per day). A similar approximation for the October sample is 13.9 increments per month (0.46 increments per day). All females in the October sample exhibited some degree of gonadal development and their statoliths had an R3 region. Since fasting has been found to be associated with sexual maturation in *Octopus vulgaris* (Wells and Wells, 1977) and in *I. illecebrosus* (personal observations), and since sexual maturation in *I. illecebrosus* can be induced by starvation (Rowe and Mangold, 1975), it is postulated that the closely-spaced increments in the R3 region may be associated with decreased food intake and maturation. Starvation is

TABLE 2. Increment and region widths (μm) and number of increments in seven statoliths from seven female *I. illecebrosus* captured in October 1981. (All other statoliths of females in the sample exhibited growth typical of R3.)

Statolith zone	Increment width		Region width			Number of increments		
	Range	Mean	Range	Mean	SD	Range	Mean	SD
Nucleus	—	—	15–21	17.8	1.5	—	—	—
R1	2.5–3.5	3.0	109–139	122.0	9.5	31–51	40.1	7.8
R2	1.5–5.0	3.4	378–431	403.0	22.5	97–140	127.1	7.9
R3	2.0–2.6	2.3	98–122	112.0	10.5	40–65	50.6	8.7

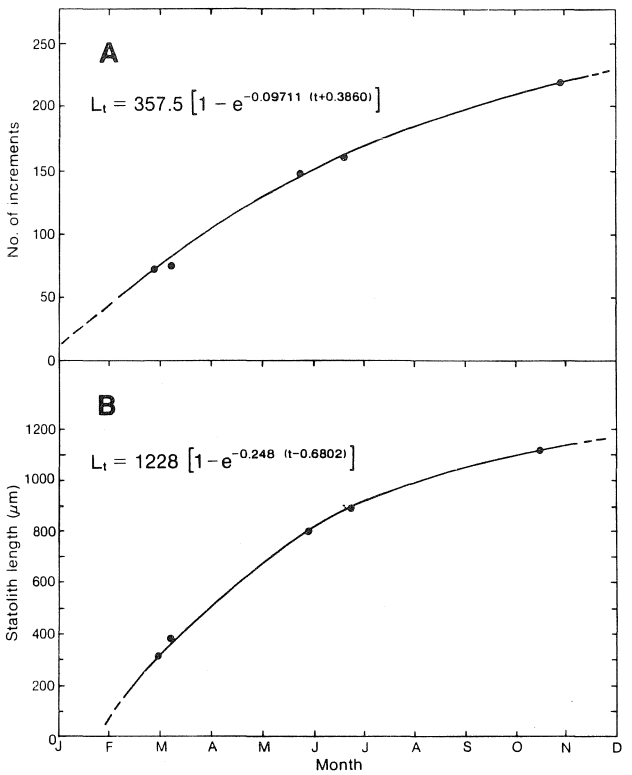

Fig. 4. Application of the von Bertalanffy growth model to data from *I. illecebrosus* statoliths to show the relationship between (**A**) number of increments and data of capture, and (**B**) statolith length and data of capture.

known to cause a narrowing of the growth increments in structures such as the shells of the clam *Mercenaria mercenaria* (Pannella and MacClintock, 1968) and the otoliths of the green sunfish *Lepomis cyanellus* (Taubert, 1975). If the increments in the statoliths are considered to represent generally some sort of a daily rhythm, the low increment counts in the statoliths of the October specimens may be due to disruption in the daily rhythm of increment formation or difficulty by the observer in resolving the smallest increments in the R3 region, or both.

From the senior author's experience with laboratory-reared *Alloteuthis subulata* and wild *Loligo* sp., hematoxylin-stained and eosin-stained statoliths of hatchlings were 80–120 and 144–205 μm in length respectively and had 18 or more increments (unpublished data). Because the statolith of *I. illecebrosus* must be large enough to be functional at hatching and because the nucleus is of such small size (Table 2), it is likely that some increments are already present at hatching in this species also. From the growth relationship in Fig. 4A, extrapolation of the curve to the x-axis indicates a date of increment formation in mid-December. If the increments in R1 (approximately 40) are considered to be prehatching increments, the predicted hatching date from Fig. 4 should be advanced by

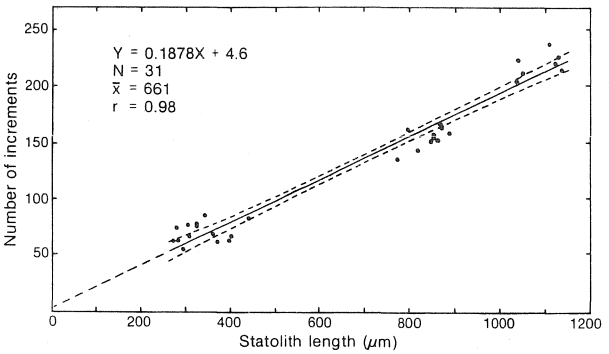

Fig. 5. Linear relationship (with 95% confidence limits) between number of increments and length of statoliths from *I. illecebrosus*.

about 40 "days" to a time in late January. Such a data closely agrees with that predicted from the relationship between stratolith length and date of capture (Fig. 4B) and that suggested by Squires (1967) from his analysis of mantle lengths. A late-January hatching time is also consistent with capture of recently-hatched *I. illecebrosus* larvae (<2 mm ML) in the Gulf Stream off the northeastern United States in late January 1982 (Hatanaka *et al.*, 1985).

The formation of the R2 region in the statolith is postulated to begin at the time of hatching. The larvae, after release from the protection of the egg mass, are exposed to environmental variations which affect the formation of increments, and this is reflected by the greater variation of both increment width and ring darkness in the R2 region than in the R1 region.

Statolith length and number of increments appear to be linearly related with the extrapolated line passing very close to the origin (Fig. 5). However, the linear regressions of increment number on mantle length for all specimens (Fig. 6A) and statolith length on mantle length (excluding juveniles) (Fig. 6B) have y-intercepts which deviate greatly from the expected value of zero. These observations imply that increment number and statolith size increase very rapidly relative to mantle length of the squid during early posthatching development.

The data for juveniles (<50 mm ML) in Fig. 6B are not consistent with the linear relationship between statolith length and mantle length, which seems to be applicable for the larger squid. The relative growth of the statolith is much greater in these juveniles than in the older animals, and the change apparently occurs at about 50 mm ML. This change is approximately coincident with a radical change in the basic shape of the statolith (Morris and Aldrich, 1984) and is presumably associated with a change in the locomotive habits of the animal. Similar observations on change in growth of the statolith relative to growth of the animal have

been noted for the Arctic squid (*Gonatus fabricii*) by Kristensen (1980) and Wiborg *et al.* (1984).

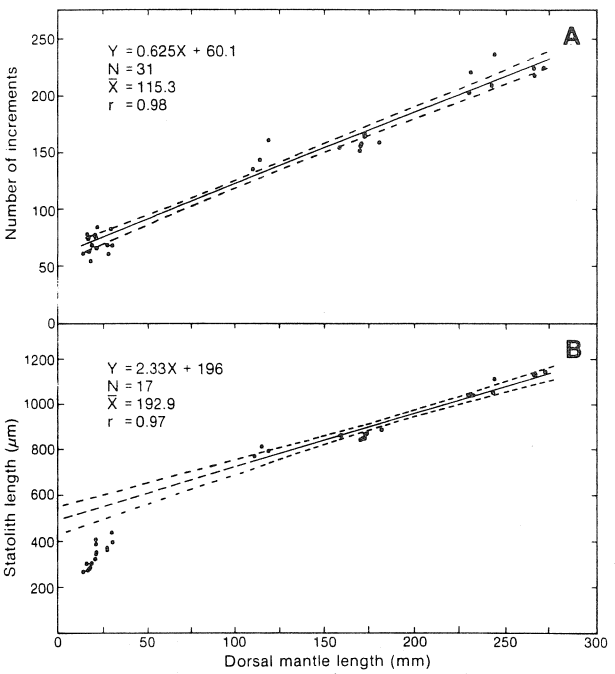

Fig. 6. Linear relationships (with 95% confidence limits) between (**A**) number of increments and mantle length for all *I. illecebrosus* specimens, and (**B**) statolith length and mantle length with juveniles (<50 mm) excluded.

Changes in the pattern of growth have frequently been used to distinguish larval and juvenile stages of fish (Nesis, 1979). Data from the present study of statoliths from *I. illecebrosus* indicate that the larval stage of ommastrephid squids may extend beyond the presently-accepted rhynchoteuthion stage which ends at 7.5–8.5 mm ML (Vecchione, 1979; Roper and Lu, 1979). Other characteristics of post-rhynchoteuthid *I. illecebrosus*, that are considered to be larval in other cephalopods (i.e. suckers along more than half the tentacular length and relative arm length not matching that of the adult (Nesis, 1979), have been noted by the authors during the course of this investigation.

Statolith length was found to vary less than number of increments in individual samples. Until better techniques are developed to examine growth increments in *I. illecebrosus*, the length of the statolith may prove to be a more reliable indicator of age than methods which involve the counting of increments.

Throughout the statolith, noticeably darker rings were often observed, with a pattern of one dark ring followed by seven or a multiple of seven lighter rings (Fig. 7). However, the dark rings were interspersed throughout this pattern. The designation of a particular dark ring as fitting this pattern was arbitrary, and, consequently, no attempt has been made to give this pattern any biological interpretation or significance.

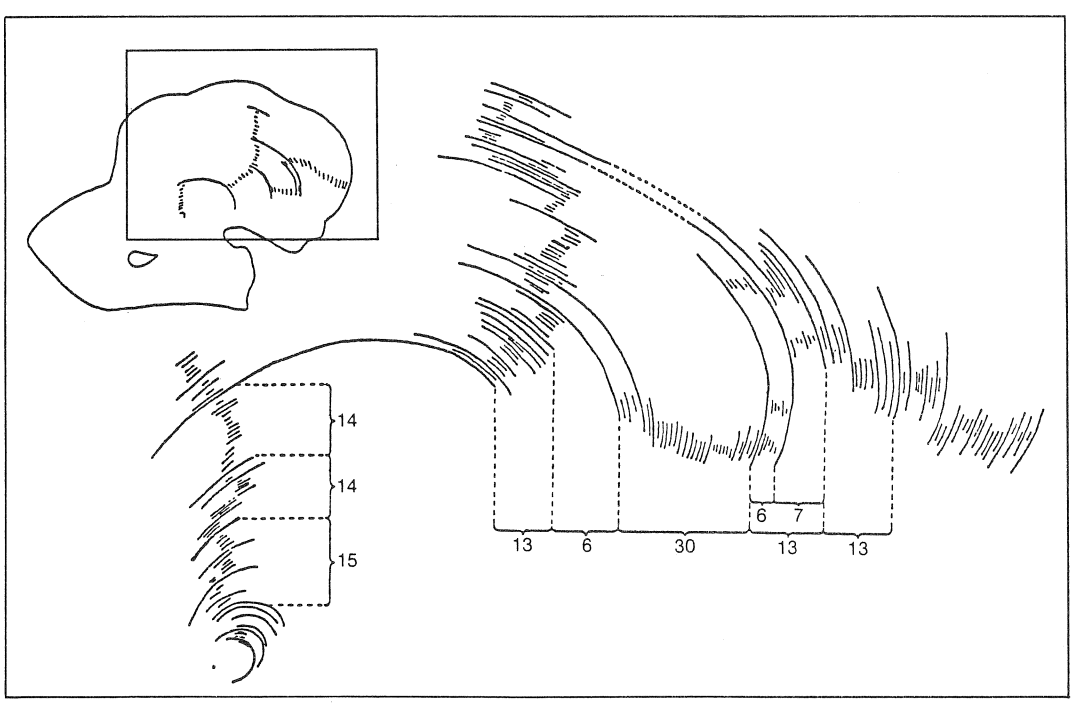

Fig. 7. Diagrammatic representation of tracing of growth rings in a statolith (997 μm long) from a male *I. illecebrosus* (212 mm ML).

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